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APPLICATION FOR UNITED STATES LETTERS PATENT

SPECIFICATION

TO ALL WHOM IT MAY CONCERN:

Be it known that we, **Peter Morris**, a citizen of Great Britain, residing at 5 Penicuik Road Roslin, Midlothian EH25 9LJ, Great Britain, and **Thomas Stiefel** a citizen of Germany, residing at Steinkopfstrasse 22, 70184 Stuttgart, Germany, and **Wolfgang Voelter**, a citizen of Germany, residing at Panoramastrasse 71, 72070 Tuebingen, Germany, and **Peter Welters**, a citizen of Germany, residing at Koelsumer Weg33, 41334 Nettetal, Germany have invented new and useful **RECOMBINANT MISTLETOE LECTINS**, of which the following is a specification.

Recombinant Mistletoe Lectins

The present invention relates to processes for the production of mistletoe lectin polypeptides in homologous and heterologous host systems and mistletoe lectin peptides as such. Further, nucleic acid molecules are provided, which code for these mistletoe lectin polypeptides, and also pharmaceutical compositions which contain these mistletoe lectin polypeptides or mistletoe lectin nucleic acids.

Mistletoe (Viscum album) has been known from antiquity as a healing plant. The semishrub plant lives as a semiparasite on the branches of woody plants and is particularly widespread in Europe, North Australia, Asia and in tropical and subtropical Africa. At the start of this century, the cyto- and tumour-toxic action of mistletoe extract, which has since then been specifically used for cancer therapy, was recognised. For this, the extract is used both as a single therapeutic agent and also in combination with chemo- or radiation therapy. Mistletoe preparations are particularly often used for example as a prophylactic against relapse after surgical tumour removal.

Systematic studies of the mode of action show that, after injection, aqueous mistletoe extract as well as its cytotoxic action also has an immunomodulatory effect, and apart from this shows generally mood-brightening effects. After injection of mistletoe extract, a significant increase in the cell numbers of certain lymphocyte subpopulations (inter alia T helper lymphocytes, natural killer (NK) cells and macrophages) and phagocytosis activity in granulo- and monocytes, which are directly involved in tumour defence, are observed (Hajto T, Hostanska K, Gabius H-J, (1990), Therapeutikum **4**, 135-145; Beuth J, Ko H L, Tunggal L, Gabius H-J, Steuer M, Uhlenbruck G, Pulverer G (1993), Med. Welt **44**, 217-220; Beuth J, Ko H L, Tunggal L, Geisel J, Pulverer G (1993), Arzneim.-Forsch/Drug Res. **43** (1), 166-169; Beuth J, Ko H L, Gabius H-J, Burricheter H, Oette Kl, Pulverer G (1992), Clin. Investing, **70**, 658-661). Further, a significant increase in defined acute phase proteins in the serum, which is mediated by the cytokines IL-1, IL-6 and TNF-α, can be detected (Hajto T, Hostanska K, Frei K, Rordorf C, Gabius H-J (1990), Cancer Res. **50**, 3322-3326; Beuth J, Ko H-L, Gabius H-J, Pulverer G (1991), In Vivo **5**, 29-32; Beuth J, Ko H-L, Tunggal L, Jeljaszewicz J, Steuer M K, Pulverer G (1994), In Vivo **8**, 989-992; Beuth J, Ko H-L, Tunggal L, Jeljaszewicz J, Steuer

M K, Pulverer G (1994), Dtsch. Zschr. Onkol. **26**, 1-6; Beuth J, Ko H-L, Tunggal L, Steuer M K, Geisel J, Jeljaszewicz J, Pulverer G (1993), In Vivo 7, 407-410; Kayser K, Gabius S, Gabius H-J, Hagemeyer O (1992) Tumordiag. und Ther. **13**, 190-195). As well as the prolongation of the survival time of cancer patients achievable by mistletoe extract treatment, an increase in the patients' quality of life is also observed, which is attributed to the rise in β-endorphins in the blood (Heiny B-M, Beuth J (1994), Anticancer Res. **14**, 1339-1342; Heiny B-M, Beuth J (1994), Dtsch. Zschr. Onkol. **26**, 103-108). As endogenous opioids, β-endorphins improve the general well-being, in that they for example have a pain-relieving action, and improve the pain index (Falconer J, Chan E C, Madsens G (1988), J. Endocrinol. **118**, 5-8).

Analysis of the active substances of mistletoe extract has shown that the immunostimulating effect is attributable to a certain group of glycoproteins, the mistletoe lectins. Hitherto, three mistletoe-specific lectins with different molecular weights and sugar-binding specificities had been identified. The concentration of mistletoe lectin I (ML-I) in the aqueous plant extract is markedly higher than that of mistletoe lectin II (ML-II) and mistletoe lectin III (ML-III). It could be shown that the immunostimulating effect of the mistletoe extract is attributable to the presence of ML-I: if the ML-I lectin is removed from the mistletoe extract, the extract loses its immunostimulating action (Beuth J, Stoffel B, Ko H-L, Jeljaszewicz J, Pulverer G (1995), Arzneim.-Forsch./Drug. Res. **45** (II), 1240-1242). The β-galactoside-specific ML-I lectin consists of two A- and two B-chains (MLA and MLB), each glycosylated, whose molecular weights are about 29 kDa and 34 kDa respectively. The amino acid sequence of MLA contains one potential glycosylation site, while MLB contains three glycosylation sites in the N-terminal region of the amino acid sequence. The two chains are linked together via a disulphide bridge (Figure A; Ziska P, Franz H, Kindt A (1978), Experientia **34**, 123-124). The resulting mistletoe lectin monomers can associate into dimers with the formation of non-covalent bonds.

Studies of the sedimentation behaviour of ML-I during analytical centrifugation show that <u>in vivo</u> ML-I is present in a monomer-dimer equilibrium (Luther P, Theise H, Chatterjee B, Kardruck D, Uhlenbruck G (1980), Int. J. Biochem. **11**, 429-435). The MLB-chain is able to bind to galactose-containing structures on the surface of cell membranes (e.g. receptor

molecules) and thereby to trigger cytokine release. Through endocytosis, ML-I dimers and monomers get into the cell, where the protein complexes break down into MLA and MLB chains through reduction of the disulphide bridge bonds. The MLA chains are thereupon able to bind to the ribosomal 28 S subunit and to inactivate this.

The study of ML-I monomers using 2-D gel electrophoresis yielded 25 different isoforms, which are attributable to different combinations of various A and B chains and different glycosylation states of the chains (Schink et al., 1992, Naturwissenschaften 79, 80-81). It is suspected that the individual isoforms fulfil specific functions and each of these isoforms contributes to the anti-tumorigenic effect of the mistletoe extract.

By now, a nucleic acid sequence and the amino acid sequence derived therefrom of one ML-I lectin is already known from European Patent Application EP 0 751 211 A1. However this one polypeptide is not capable of satisfactorily emulating the action of the many ML-I isoenzymes contained in natural mistletoe extract as regards the anti-tumorigenic and mood-brightening effect.

Hence the technical problem of the present invention is to provide a process which makes it possible to produce mistletoe lectins in sufficient quantities and at the same time to imitate the diversity in ML-I isoenzymes of the natural mistletoe extract.

The problem is solved according to the invention by the provision of a process according to Claim 1 and/or 40.

The present invention moreover makes available 2 new polypeptides of the MLA chain and 6 new polypeptides of the MLB chain of ML-I, which can be expressed individually or in combination in a suitable host system. Thereby, “homologous” and "heterologous" ML-I dimers are formed, where the term “homologous” denotes a dimer which consists either of two MLA and two MLB chains, each the same and the term “heterologous” denotes a dimer which consists of two different MLA and/or two different MLB chains. The diversity of the MLA and MLB chains makes it possible to create a multitude of different MLA/MLB complexes, the therapeutic action of which is modelled on the above-described action of the

lectin mixture which was detected in aqueous mistletoe extract. One of the advantages which the present invention offers compared to the conventional extraction of mistletoe extracts from fresh plants is that the immunomodulating components of the mistletoe extract can be produced by a biotechnological process. This means that sufficient quantities of mistletoe lectin I can be produced independently of plant material, which is only available to a limited extent and can only be harvested at a certain time of year. Furthermore, a mixture of mistletoe lectins biotechnologically produced in this way contains none of the "impurities" occurring in the natural mistletoe extract, e.g. viscotoxins.

Further, owing to the fact that the present invention makes a large number of different MLA and MLB polypeptides of ML-I available, it becomes possible to "design" pharmacological compositions in a target-oriented manner. This means that e.g. by the selection of certain MLB polypeptides which define the binding affinity of the MLA/MLB complex to the target cells, the immunomodulatory action of a composition can be influenced. Furthermore, by the use of defined MLA polypeptides, the cytotoxicity of a composition can be varied.

In order to be able biotechnologically to produce the mixture of mistletoe lectins contained in mistletoe extracts, firstly the amino acid sequence of a pharmaceutically interesting mistletoe lectin was elucidated. For this, a mistletoe extract was obtained from *Viscum album L. ssp. platyspermum* Kell, which were harvested from poplars, and mistletoe lectin I was partially purified by affinity chromatography (Example 1). The subsequent analysis by SDS-PAGE, HPLC and sequence analysis by Edman degradation showed 2 MLA isoforms and 6 MLB isoforms.

Degenerate oligonucleotides were derived from short regions of the amino acid sequences, and by means of these the genomic mistletoe lectin I DNA sequence was determined using the PCR process. Surprisingly, in spite of the many identified ML-I amino acid sequences, only a single nucleic acid sequence more less corresponding to these sequences was identified. By Southern blot analysis, it was confirmed that the ML-I gene occurs in only one copy per genome. Hence the sequence variability of the MLA and MLB polypeptides is to be explained only by the occurrence of RNA editing or other posttranscriptional or posttrans-lational modifications in mistletoe cells.

All processes that lead to differences between the final mRNA sequence and the corresponding "template" DNA, except for "RNA-splicing" and tRNA modifications, are described as "RNA-editing". "mRNA-splicing", and also the occurrence of modified tRNAs, is generally known and is therefore not explained in more detail here. In "RNA-editing", individual nucleotides or strands of up to several hundred nucleotides in length are exchanged, inserted or deleted co- or posttranscriptionally, which can lead to reading-frame changes in the coded sequence. The first example of RNA-editing was discovered in studies of the coxII transcript of the mitochondrial DNA of trypansomes (Benne R et al (1986) Cell **46**, 819-826). Further, this process has been detected in mitochondria and chloroplasts of higher plants and singular nuclear transcripts in mammalian cells. The precise mechanism of RNA editing, like the mechanisms for posttranslational modifications of the primary amino acid sequence have however so far only been very incompletely described in the literature.

Since however this process has so far only been detected in very few plants and the aim is to make biotechnological production of the various mistletoe lectin I polypeptides also possible in other plant cells than mistletoe cells as far as possible independently of posttranscriptional or posttranslational changes, the genomic DNA was matched to the sequence of the various isolated polypeptides by deliberate mutations. Furthermore, the genomic sequence was matched to the preferred codon utilisation of *Brassica*, in order to make optimal expression possible e.g. in rape cells.

~~Hence the present invention makes available a process for the production of a mistletoe lectin polypeptide or a fragment thereof in the heterologous system having the following sequence:~~

Y E R L R L R V T H Q T T G X1 E Y F R F I T L

L R D Y V S S G S F S N E I P L L R Q S T I P

V S D A Q R F V L V E L T N Q G X2 D S X3 T A A

I D V T N X4 Y V V A Y Q A G D Q S Y F L R D A

P R G A E T H L F T G T T R X5 S S L P F X6 G S

Y X7 D L E R Y A G H R D Q I P L G I X8 Q L I Q

S V X9 A L R X10 P G G S T R X11 Q A R S I L I L

I Q M I S E A A R F N P I L W R X12 R Q X13 I N

S G X14 S F L P D X15 Y M L E L E T S W G Q Q S

T Q V Q H S T D G V F N N P X16 R L A I X17 X18 G

N F V T L X19 N V R X20 V I A S L A I M L F V C

G E R P S S S D V R Y W P L V I R P V I A D D

V T C S A S E P T V R I V G R X21 G M X22 V D V

R D D D F H D G N Q I Q L W P S K S N N D P N

Q L W T I K R D X23 T I R S N G S C L T T Y G Y

T A G V Y V M I F D C N T A V R E A T I W Q I

W X24 N G T I I N P R S N L V L A A S S G I K G

T T L T V Q T L D Y T L G Q G W L A G N D T A

P R E V T I Y G F R D L C M E S N X25 G S V W V

E T C X26 S S Q X27 N Q X28 X29 W A L Y G D G S I R

P K Q N Q D Q C L T X30 G R D S V S T V I N I V

S C S X31 X32 S X33 X34 Q R W V F T N E X35 A I L N

L K X36 X37 X38 X39 X40 D V A Q A N P K L R R I I I

Y P A T G K P N Q M W L P V X41

including the step of expressing of a eukaryotic or prokaryotic vector, into which a nucleic acid coding for the mistletoe lectin polypeptide according to the usual genetic code or a fragment thereof is cloned, in a suitable heterologous eukaryotic or prokaryotic host,

wherein X1 is D or E, X2 is G or Q, X3 is I or V, X4 is L or A, X5 is DR or missing, X6 is N or T, X7 is P or T, X8 is D or E, X9 is S or T, X10 is F or Y, X11 is T or A, X12 is A or Y, X13 is Y or D, X14 is A or E, X15 is V or M, X16 is I or F, X17 is P or S, X18 is P or T, X19 is T or S, X20 is D or S, X21 is N or S, X22 is C or R, X23 is G or N, X24 is G or D,

X25 is G or Q, X26 is V or D, X27 is Q or K, X28 is G or missing, X29 is R or K, X30 is C or S or V, X31 is A or G, X32 is G or A, X33 is S or G, X34 is G or S, X35 is G or Y, X36 is N or S or T or K, X37 is S or G, X38 is L or P, X39 is A or M, X40 is M or V and X41 is P or F.

~~Analogously to this process, two further production processes for the mistletoe lectin A-chain~~ (MLA) and mistletoe lectin B-chain (MLB) are made available, which contain the following ~~sequences or a fragment thereof.~~

Mistletoe Lectin A:

Y E R L R L R V T H Q T T G X1 E Y F R F I T L

L R D Y V S S G S F S N E I P L L R Q S T I P

V S D A Q R F V L V E L T N Q G X2 D S X3 T A A

I D V T N X4 Y V V A Y Q A G D Q S Y F L R D A

P R G A E T H L F T G T T R X5 S S L P F X6 G S

Y X7 D L E R Y A G H R D Q I P L G I X8 Q L I Q

S V X9 A L R X10 P G G S T R X11 Q A R S I L I L

I Q M I S E A A R F N P I L W R X12 R Q X13 I N

S G X14 S F L P D X15 Y M L E L E T S W G Q Q S

T Q V Q H S T D G V F N N P X16 R L A I X17 X18 G

N F V T L X19 N V R X20 V I A S L A I M L F V C

G E R P S S S

Mistletoe Lectin B:

D D V T C S A S E P T V R I V G R X21 G M X22 V D

V R D D D F H D G N Q I Q L W P S K S N N D P N

Q L W T I K R D X23 T I R S N G S C L T T Y G Y

T A G V Y V M I F D C N T A V R E A T I W Q I W

X24 N G T I I N P R S N L V L A A S S G I K G T T

L T V Q T L D Y T L G Q G W L A G N D T A P R E

V T I Y G F R D L C M E S N X25 G S V W V E T C

X26 S S Q X27 N Q X28 X29 W A L Y G D G S I R P K Q N

Q D Q C L T X30 G R D S V S T V I N I V S C S X31

X32 S X33 X34 Q R W V F T N E X35 A I L N L K X36 X37

X38 X39 X40 D V A Q A N P K L R R I I I Y P A T G

K P N Q M W L P V X41

wherein X1 to X41 have the meaning stated above.

~~Furthermore, a mistletoe lectin polypeptide or a fragment thereof, which includes the sequence variability of the various MLA and MLB chains, having the following sequence is provided:~~

Y E R L R L R V T H Q T T G X1 E Y F R F I T L

L R D Y V S S G S F S N E I P L L R Q S T I P

V S D A Q R F V L V E L T N Q G X2 D S X3 T A A

I D V T N X4 Y V V A Y Q A G D Q S Y F L R D A

P R G A E T H L F T G T T R X5 S S L P F X6 G S

Y X7 D L E R Y A G H R D Q I P L G I X8 Q L I Q

S V X9 A L R X10 P G G S T R X11 Q A R S I L I L

I Q M I S E A A R F N P I L W R X12 R Q X13 I N

S G X14 S F L P D X15 Y M L E L E T S W G Q Q S

T Q V Q H S T D G V F N N P X16 R L A I X17 X18 G

N F V T L X19 N V R X20 V I A S L A I M L F V C

G E R P S S S D V R Y W P L V I R P V I A D D

V T C S A S E P T V R I V G R X21 G M X22 V D V

R D D D F H D G N Q I Q L W P S K S N N D P N

Q L W T I K R D X23 T I R S N G S C L T T Y G Y

T A G V Y V M I F D C N T A V R E A T I W Q I

W X24 N G T I I N P R S N L V L A A S S G I K G

T T L T V Q T L D Y T L G Q G W L A G N D T A

P R E V T I Y G F R D L C M E S N X25 G S V W V

E T C X26 S S Q X27 N Q X28 X29 W A L Y G D G S I R

P K Q N Q D Q C L T X30 G R D S V S T V I N I V

S C S X31 X32 S X33 X34 Q R W V F T N E X35 A I L N

L K X36 X37 X38 X39 X40 D V A Q A N P K L R R I I I

Y P A T G K P N Q M W L P V X41

~~Apart from this, mistletoe lectin polypeptides of the mistletoe lectin A-chain and mistletoe~~ lectin B-chain or ~~fragments~~ of these sequences are provided, which include the following ~~sequences:~~

Mistletoe Lectin A:

Y E R L R L R V T H Q T T G X1 E Y F R F I T L

L R D Y V S S G S F S N E I P L L R Q S T I P

V S D A Q R F V L V E L T N Q G X2 D S X3 T A A

I D V T N X4 Y V V A Y Q A G D Q S Y F L R D A

P R G A E T H L F T G T T R X5 S S L P F X6 G S

Y X7 D L E R Y A G H R D Q I P L G I X8 Q L I Q

S V X9 A L R X10 P G G S T R X11 Q A R S I L I L

I Q M I S E A A R F N P I L W R X12 R Q X13 I N

S G X14 S F L P D X15 Y M L E L E T S W G Q Q S

T Q V Q H S T D G V F N N P X16 R L A I X17 X18 G

N F V T L X19 N V R X20 V I A S L A I M L F V C

G E R P S S S

Mistletoe Lectin B:

D D V T C S A S E P T V R I V G R X21 G M X22 V D

V R D D D F H D G N Q I Q L W P S K S N N D P N

Q L W T I K R D X23 T I R S N G S C L T T Y G Y

T A G V Y V M I F D C N T A V R E A T I W Q I W

X24 N G T I I N P R S N L V L A A S S G I K G T T

L T V Q T L D Y T L G Q G W L A G N D T A P R E

V T I Y G F R D L C M E S N X25 G S V W V E T C

X26 S S Q X27 N Q X28 X29 W A L Y G D G S I R P K Q N

Q D Q C L T X30 G R D S V S T V I N I V S C S X31

X32 S X33 X34 Q R W V F T N E X35 A I L N L K X36 X37

X38 X39 X40 D V A Q A N P K L R R I I I Y P A T G

K P N Q M W L P V X41

wherein X1 to X41 have the meaning stated above.

The sequence which includes the above-described variability of the ML-I polypeptides occurring in mistletoe cells is shown in Figure 1b. A specific sequence for MLA2 of mistletoe lectin I, which was likewise produced according to the process presented above, is shown in Figure 3b. Figures 7b to 12b include specific mistletoe lectin B-chain sequences, which were likewise produced according to the process described above.

A further aspect of the present invention is a process for the provision of a nucleic acid molecule, which codes for a mistletoe lectin polypeptide in a heterologous host as described above and includes the following steps:

a) preparing of mistletoe cell RNA or chromosomal mistletoe cell DNA and

b) amplifying mistletoe cell RNA or chromosomal mistletoe lectin DNA by PCR using oligonucleotides which are derived from the mistletoe lectin polypeptide shown in Fig.1b, and

c) if necessary, identifying of sequences which lie 5' and 3' from the amplified nucleic acid and amplification thereof, and

d) isolating of the nucleic acid molecules amplified in step b) and/or c), and

e) if necessary, ligating of several of the nucleic acid molecules amplified in step b) and/or c), such that a nucleic acid molecule with a complete open reading frame is obtained and

f) targeted mutation of the nucleic acid molecule obtained in order to match the nucleic acid molecule to the usual genetic code of the heterologous host for one of the mistletoe lectin polypeptide isoforms identified in mistletoe cells.

For the preparation of mistletoe cell DNA, mistletoe plants (*Viscum album L. ssp. platy-spermum* Kell), which had been harvested from poplars from Alsace, were crushed in liquid nitrogen and the chromosomal DNA extracted (Example 1). Using the degenerate oligo-nucleotides shown below, fragments of the genomic mistletoe lectin DNA were amplified by means of the PCR process (Example 2). The degenerate oligonucleotides used in the PCR reaction, which hybridise to regions of the MLB chain DNA, have the sequence:

(BI):

GTN MGN GAY GAY GAY TTY CA

(BII):

AT YTG RTT NGG YTT NCC NGT

The abbreviations of the nucleotides here are based on the designation proposed by the IUPAC-IUB Biochemical Nomenclature Commission.

In a further reaction step, using specific oligonucleotides, the 5'- and 3'-lying sequences of the first amplification product were determined by means of the RACE technique (Example 3). The oligonucleotide used for the 5'-RACE reaction has the following sequence:

CAC AGC AGT ATT ACA GTC GAA.

The oligonucleotide used for the 3'-RACE reaction has the following sequence:

GTC TAT GTG ATG ATC TTC GAC TGT.

The complete nucleic sequence thus obtained was used for the synthesis of specific oligonucleotides in order to obtain a whole clone by means of the PCR. Alternatively, the partly overlapping clones were cleaved using suitable restriction cleavage sites, in order to be assembled in a suitable vector, so that a complete open reading frame of the mistletoe lectin I gene was obtained. Deliberate mutations can be introduced into these DNA constructs by known techniques, e.g. by replacement of certain DNA regions by other DNA fragments, introduction of not completely homologous oligonucleotides, etc. These mutations can serve on the one hand to modify the amino acid sequence derived therefrom and thus to influence the activity of the polypeptide, or on the other hand to vary the nucleic acid sequence, without modifying the amino acid sequence, in order e.g. to imitate the preferred codon usage of a host organism.

Nucleic acid molecules which are made available by this process and code for a polypeptide as described above, include the following sequences for ML-I, MLA and MLB or fragments thereof:

1) ML-I Sequence

TACGAGAGGCTAAGACTCAGAGTTACGCATCAAACCACGGGCGAKGAATACTTCCGGTTCATCACG

CTTCTCCGAGATTATGTCTCAAGCGGAAGCTTTTCCAATGAGATACCACTCTTGCGTCAGTCTACG

ATCCCCGTCTCCGATGCGCAAAGATTTGTCTTGGTGGAGCTCACCAACCAGGGGSRRGACTCGRTY

ACGGCCGCCATCGACGTTACCAATSYKTACGTCGTGGCTTACCAAGCAGGCGACCAATCCTACTTT

TTGCGCGACGCACCACGCGGCGCGGAAACGCACCTCTTCACCGGCACCACCCGAZ1TCCTCTCTCC

CATTCAMYGGAAGCTACMCYGATCTGGAGCGATACGCCGGACATAGGGACCAGATCCCTCTCGGTA

TAGASCAACTCATTCAATCCGTCWCKGCGCTTCGTTWYCCGGGCGGCAGCACGCGTRCYCAAGCTC

GTTCGATTTTAATCCTCATTCAGATGATCTCCGAGGCCGCCAGATTCAATCCCATCTTATGGAGGK

MYCGCCAAKAYATTAACAGTGGGGMRTCATTTCTGCCAGACRTGTACATGCTGGAGCTGGAGACGA

GTTGGGGCCAACAATCCACGCAAGTCCAGCATTCAACCGATGGCGTTTTTAATAACCCAWTYCGGT

TGGCTATAYCYMCYGGTAACTTCGTGACGTTGWCYAATGTTCGCKMYGTGATCGCCAGCTTGGCGA

TCATGTTGTTTGTATGCGGAGAGCGGCCATCTTCCTCTGACGTGCGCTATTGGCCGCTGGTCATAC

GACCCGTGATAGCCGATGATGTTACCTGCAGTGCTTCGGAACCTACGGTGCGGATTGTGGGTCGAA

RTGGCATGYGCGTGGACGTCCGAGATGACGATTTCCACGATGGGAATCAGATACAGTTGTGGCCCT

CCAAGTCCAACAATGATCCGAATCAGTTGTGGACGATCAAAAGGGATRRMACCATTCGATCCAATG

GCAGCTGCTTGACCACGTATGGCTATACTGCTGGCGTCTATGTGATGATCTTCGACTGTAATACTG

CTGTGCGGGAGGCCACTATTTGGCAGATATGGGRCAATGGGACCATCATCAATCCAAGATCCAATC

TGGTTTTGGCAGCATCATCTGGAATCAAAGGCACTACGCTTACGGTGCAAACACTGGATTACACGT

TGGGACAGGGCTGGCTTGCCGGTAATGATACCGCCCCACGCGAGGTGACCATATATGGTTTCAGGG

ACCTTTGCATGGAATCAAATSRAGGGAGTGTGTGGGTGGAGACGTGCGWSAGTAGCCAAMAGAACC

AAZ2ARATGGGCTTTGTACGGGGATGGTTCTATACGCCCCAAACAAAACCAAGACCAATGCCTCAC

CKBTGGGAGAGACTCCGTTTCAACAGTAATCAATATAGTTAGCTGCAGCGSWGSWTCGKSKKSKCA

GCGATGGGTGTTTACCAATGAAKRSGCCATTTTGAATTTAAAGAVWRGSYYGRYSRTGGATGTGGC

GCAAGCAAATCCAAAGCTCCGCCGAATAATTATCTATCCTGCCACAGGAAAACCAAATCAAATGTG

GCTTCCCGTGYYMTGA

II) MLA Sequence

TACGAGAGGCTAAGACTCAGAGTTACGCATCAAACCACGGGCGAKGAATACTTCCGGTTCATCACG

CTTCTCCGAGATTATGTCTCAAGCGGAAGCTTTTCCAATGAGATACCACTCTTGCGTCAGTCTACG

ATCCCCGTCTCCGATGCGCAAAGATTTGTCTTGGTGGAGCTCACCAACCAGGGGSRRGACTCGRTY

ACGGCCGCCATCGACGTTACCAATSYKTACGTCGTGGCTTACCAAGCAGGCGACCAATCCTACTTT

TTGCGCGACGCACCACGCGGCGCGGAAACGCACCTCTTCACCGGCACCACCCGAZ1TCCTCTCTCC

CATTCAMYGGAAGCTACMCYGATCTGGAGCGATACGCCGGACATAGGGACCAGATCCCTCTCGGTA

TAGASCAACTCATTCAATCCGTCWCKGCGCTTCGTTWYCCGGGCGGCAGCACGCGTRCYCAAGCTC

GTTCGATTTTAATCCTCATTCAGATGATCTCCGAGGCCGCCAGATTCAATCCCATCTTATGGAGGK

MYCGCCAAKAYATTAACAGTGGGGMRTCATTTCTGCCAGACRTGTACATGCTGGAGCTGGAGACGA

GTTGGGGCCAACAATCCACGCAAGTCCAGCATTCAACCGATGGCGTTTTTAATAACCCAWTYCGGT

TGGCTATAYCYMCYGGTAACTTCGTGACGTTGWCYAATGTTCGCKMYGTGATCGCCAGCTTGGCGA

TCATGTTGTTTGTATGCGGAGAGCGGCCATCTTCCTCT

III) MLB Sequence

GATGATGTTACCTGCAGTGCTTCGGAACCTACGGTGCGGATTGTGGGTCGAARTGGCATGYGCGTG

GACGTCCGAGATGACGATTTCCACGATGGGAATCAGATACAGTTGTGGCCCTCCAAGTCCAACAAT

GATCCGAATCAGTTGTGGACGATCAAAAGGGATRRMACCATTCGATCCAATGGCAGCTGCTTGACC

ACGTATGGCTATACTGCTGGCGTCTATGTGATGATCTTCGACTGTAATACTGCTGTGCGGGAGGCC

ACTATTTGGCAGATATGGGRCAATGGGACCATCATCAATCCAAGATCCAATCTGGTTTTGGCAGCA

TCATCTGGAATCAAAGGCACTACGCTTACGGTGCAAACACTGGATTACACGTTGGGACAGGGCTGG

CTTGCCGGTAATGATACCGCCCCACGCGAGGTGACCATATATGGTTTCAGGGACCTTTGCATGGAA

TCAAATSRAGGGAGTGTGTGGGTGGAGACGTGCGWSAGTAGCCAAMAGAACCAAZ2ARATGGGCTT

TGTACGGGGATGGTTCTATACGCCCCAAACAAAACCAAGACCAATGCCTCACCKBTGGGAGAGACT

CCGTTTCAACAGTAATCAATATAGTTAGCTGCAGCGSWGSWTCGKSKKSKCAGCGATGGGTGTTTA

CCAATGAAKRSGCCATTTTGAATTTAAAGAVWRGSYYGRYSRTGGATGTGGCGCAAGCAAATCCAA

AGCTCCGCCGAATAATTATCTATCCTGCCACAGGAAAACCAAATCAAATGTGGCTTCCCGTGYYMT

GA

The nucleotides are defined in accordance with the IUPAC-IUB code; Z_1 designates the nucleotide sequence GAT AGA or is missing, while Z_2 designates the nucleotide GGC or is missing.

~~DNA specific nucleic acid molecule which was prepared by the process stated above and includes the entire ML-I coding sequence, is shown in Figure 1a. Further specific nucleic acid molecules, which code for the MLA chain of mistletoe lectin I and were prepared by the process stated above, are shown in Figure 2a and Figure 2b. Specific sequences for MLB nucleic acid molecules, which were prepared by the process described above, are listed in~~

Figures 7a to 12 a. Here, each of these nucleic acid sequences codes for a polypeptide which emerged by protein sequencing of the ML-I mixture from natural mistletoe extract.

In addition, the present invention includes nucleic acid molecules which code for a mistletoe lectin polypeptide, as described above, and are characterised in that the codon usage is matched to the requirements of a heterologous host. Figure 4a shows such a nucleic acid sequence, wherein the codon usage is matched to the preferred codon usage of the genus *Brassica*. This genus was chosen, since both as the Summer and also as the Winter form it thrives outstandingly in the middle latitudes of Europe, North America and Asia. The possible uses of rape for the production of recombinant proteins have been demonstrated by various firms and research institutes. Examples of its use are the production of gastric lipase for use in the treatment of cystic fibrosis or coupling to oleosins for greater ease of purification of the recombinant proteins from the lipid phase of the rape oil seeds.

The sequences shown in Figures 5a, 6a and 13a to 18a represent nucleic acid molecules which code for MLA polypeptides or for MLB polypeptides of mistletoe lectin I and whose codon usage is likewise matched to the genus *Brassica*. The degree of homology between these matched sequences to the nucleic acid sequences shown in Figs. 2a and 7a is ca. 61% for MLA and about 63% for MLB.

Further, through the present invention a vector is made available, which includes one of the nucleic acid molecules described above or a fragment thereof and also a promoter regulating the expression of this nucleic acid molecule. In a preferred embodiment, this vector contains, in functional linkage with the nucleic acid molecules described above, a promoter which can only be activated in the intended host cell. The host cell here can be a plant or an animal cell. Host-specific promoters are already used, sometimes together with cell type-specific, regulated enhancer sequences, for the selective expression of therapeutic genes (Walter W and Stein U, Molecular Biotechnology, 1996, **6** (3), 267-86). Likewise, systems have been developed, wherein inducers and repressors act on a genetically modified transcription factor, which specifically recognises a likewise modified promoter. This allows the regulated expression of e.g. therapeutic proteins, without at the same time non-specifically activating cellular promoters (Miller N and Whelan J, Human Gene Therapy, 1997, **8** (7), 803-815).

A preferred vector is an RNA vector, such as for example described in Kumagai et al., Proc. Natl. Acad. Sci., USA, 1993, **90**, 427-430. Compared to other plant expression systems, this system offers the advantages firstly that high yields of recombinant proteins can be achieved and secondly a considerably faster establishment of the process takes place, since only the RNA vector is genetically modified, and after infection the plant starts the production of the recombinant protein.

Host systems which are to serve for the heterologous expression of the nucleic acids described above can be selected from the group including bacterial cells, plant cells with the exception of mistletoe cells, insect cells, insect larvae, vertebrate cells, preferably mammalian cells, yeast cells, fungal cells, transgenic vertebrates with the exception of man and/or transgenic plants with the exception of mistletoe plants. Here preferably *Escherichia coli* are used as bacterial cells, rape cells as plant cells, Trichoplusia ni as insect larvae, *Spodoptera frugiperda* cells as insect cells and zebra fish as vertebrates.

The present invention includes pharmaceutical compositions which contain at least one of the aforementioned nucleic acid molecules or one of the vectors described above.

A preferred pharmaceutical composition in addition contains liposomes, which enclose the linear nucleic acid molecules or the vectors, in order to protect them against nucleolytic degradation. At the same time, these liposomes can bear cell recognition molecules on their surface, which enable selective attachment to specific target cells. Such so-called "second generation" surface-modified liposomes (e.g. immunoliposomes and "long-circulating liposomes") are already being successfully used for the targeted transfection of specific cell types from cancer patients (Storm G and Crommelin D J, Hybridoma, 1997, **16** (1), 119-125, Thierry A R et al., Gene Therapy, 1997, **4** (3), 226-237).

A further pharmaceutical composition is specified, wherein the linear nucleic acid molecule or the vector is coupled directly or via a linker system (e.g. biotin-streptavidin coupling) to one of the MLB polypeptides described above. Here the MLB polypeptide unit mediates the attachment of the complex to sugar-containing structures on the cell membrane and induces the endocytotic uptake of the complex. In this way, for example a nucleic acid coding for the

cytotoxic MLA can be specifically transported into a cell, where it is subsequently translated into a protein and then inactivates the cell's own ribosomes. In addition, such a complex can contain peptides such as for example antibodies, antibody fragments or receptor-binding peptides (ligands), which are capable of effecting cell-specific binding.

A further preferred pharmacological composition includes a virus particle, as well as the linear nucleic acid molecule or the vector. In this case, a virus vector is preferred. Here the virus particle can likewise on its surface bear cell recognition molecules for specific cell recognition. These molecules can be e.g. fusion proteins of viral proteins with cell-specific-ally binding polypeptides. By presentation of these peptides on the surface of the virus particle, a targeted attachment of these particles can be achieved (Joelson T et al., Journal of General Virology, **78** (6), 1213-1217, Grabherr R et al., Biotechnics, 1997, **22** (4), 730-735).

The present invention further includes a pharmaceutical composition which contains at least one of the mistletoe lectin polypeptides described above and/or at least one fragment thereof as cytotoxic component. The pharmaceutical efficiency of such a composition can once again be heightened by coupling of the polypeptides or the polypeptide fragments with cell recognition molecules which bind selectively to target cells. In a preferred embodiment of the pharma-ceutical composition, the cell recognition molecule is an antibody molecule, an antibody fragment or any other protein and peptide molecule, which has the capacity specifically to bind to the target cells, e.g. a peptide hormone or a fragment of this hormone such as the "gonadotropin-releasing hormone" and such fragments which specifically bind to receptors of adenocarcinoma cells or peptides which in a specific form of leukaemia bind to the inter-leukin-2 growth factor of the lymphoma cells ("cutaneous T cell lymphoma"). Non-protein molecules which concentrate in target cells or bind to them, such as cis-platin or haem and precursors thereof, can be also suitable cell recognition molecules for coupling to the cyto-toxic component of the ML-I. Owing to the fact that the cytotoxic component specifically gets into the cell interior of the degenerated cells, the dose of toxin can be kept relatively low and side-effects on healthy tissue minimised.

Here these cell recognition molecules can be coupled to the mistletoe lectin polypeptides by known chemical processes. Furthermore, it is possible to create fusion proteins from the

polypeptides described above and a suitable antibody or a fragment thereof in one of the host systems likewise described above. Also suitable as fusion proteins are e.g. recombinant proteins which consist of a polypeptide described here and an IL-2 receptor-binding "homing" component or a genetically modified fragment of gonadotropin-releasing factor.

A pharmaceutical composition according to the invention contains at least one of the polypeptides described above and/or a fragment thereof, as a rule together with a pharmaceutically compatible vehicle. Here a defined mixture of different MLA and/or MLB polypeptides corresponding to the needs of the patient can be composed. In order to recreate the diversity of the mistletoe lectin I isoenzyme of natural mistletoe extract, a cytotoxic composition preferably contains several or all of the above-stated MLA/MLB polypeptides. The pharmaceutically tolerable carrier can be a buffer, a diluent, a filler, solvent, lubricant, flavouring, binder, preservative and/or occluding material. The pharmaceutical composition is formulated such that it is suitable both for oral and also parenteral administration, in particular subcutaneous, intramuscular and intravenous administration. In certain diseases, inhalational, rectal, vaginal and cutaneous presentations can also be used.

On account of an anti-tumorigenic action, an above-mentioned mistletoe lectin polypeptide or a fragment thereof can be used for production of a medicament for treatment of uncontrolled cell growth, e.g. of cancer. Furthermore, such a mistletoe lectin polypeptide or a fragment thereof, whose cytotoxic activity has been blocked, e.g. by modifications at the active centre (amino acids Y_{76}, Y_{115}, E_{165}, R_{168}, W_{199}), in combination with at least one further antigen, can be used for the production of a medicament, which is capable of intensifying the immune reaction against the further antigen. For example, from European Patent 0 320 528, proteins are already known (haemocyanins and arylphorins), which can cause a strong antigenic reaction. Similarly to these substances, the mistletoe lectins according to the invention can also trigger an activation of T-lymphocytes and lymphokine-producing macrophages and as a result strengthen the endogeneous defences.

Furthermore, the present invention also includes a process for the production of a mistletoe lectin polypeptide in mistletoe cells and/or transgenic mistletoe plants having the following sequence:

Y E R L R L R V T H Q T T G X1 E Y F R F I T L

L R D Y V S S G S F S N E I P L L R Q S T I P

V S D A Q R F V L V E L T N Q G X2 D S X3 T A A

I D V T N X4 Y V V A Y Q A G D Q S Y F L R D A

P R G A E T H L F T G T T R X5 S S L P F X6 G S

Y X7 D L E R Y A G H R D Q I P L G I X8 Q L I Q

S V X9 A L R X10 P G G S T R X11 Q A R S I L I L

I Q M I S E A A R F N P I L W R X12 R Q X13 I N

S G X14 S F L P D X15 Y M L E L E T S W G Q Q S

T Q V Q H S T D G V F N N P X16 R L A I X17 X18 G

N F V T L X19 N V R X20 V I A S L A I M L F V C

G E R P S S S D V R Y W P L V I R P V I A D D

V T C S A S E P T V R I V G R X21 G M X22 V D V

R D D D F H D G N Q I Q L W P S K S N N D P N

Q L W T I K R D X23 T I R S N G S C L T T Y G Y

T A G V Y V M I F D C N T A V R E A T I W Q I

W X24 N G T I I N P R S N L V L A A S S G I K G

T T L T V Q T L D Y T L G Q G W L A G N D T A

P R E V T I Y G F R D L C M E S N X25 G S V W V

E T C X26 S S Q X27 N Q X28 X29 W A L Y G D G S I R

P K Q N Q D Q C L T X30 G R D S V S T V I N I V

S C S X31 X32 S X33 X34 Q R W V F T N E X35 A I L N

L K X36 X37 X38 X39 X40 D V A Q A N P K L R R I I I

Y P A T G K P N Q M W L P V X41

comprising the step of expressing a eukaryotic vector, which contains a nucleic acid coding for the mistletoe lectin polypeptide or a fragment thereof having the nucleic acid sequence originally found in mistletoe cell DNA, in a mistletoe cell or a transgenic mistletoe plant, wherein the transcription product of this nucleic acid molecule is modified in mistletoe cells or transgenic mistletoe plants by RNA editing and further normally occurring postranscript-ional and/or posttranslational mechanisms and thus possibly leads to the production of the natural mistletoe lectin mixture,

wherein X1 is D or E, X2 is G or Q, X3 is I or V, X4 is L or A, X5 is DR or missing, X6 is N or T, X7 is P or T, X8 is D or E, X9 is S or T, X10 is F or Y, X11 is T or A, X12 is A or Y, X13 is Y or D, X14 is A or E, X15 is V or M, X16 is I or F, X17 is P or S, X18 is P or T, X19 is T or S, X20 is D or S, X21 is N or S, X22 is C or R, X23 is G or N, X24 is G or D, X25 is G or Q, X26 is V or D, X27 is Q or K, X28 is G or missing, X29 is R or K, X30 is C or S or V, X31 is A or G, X32 is G or A, X33 is S or G, X34 is G or S, X35 is G or Y, X36 is N or S or T or K, X37 is S or G, X38 is L or P, X39 is A or M, X40 is M or V and X41 is P or F.

On the basis of the process described above, two further production processes for the mistletoe lectin A-chain and mistletoe lectin B-chain or a fragment thereof are provided, which contain the following sequences or a fragment thereof:

Mistletoe Lectin A:

Y E R L R L R V T H Q T T G X1 E Y F R F I T L

L R D Y V S S G S F S N E I P L L R Q S T I P

V S D A Q R F V L V E L T N Q G X2 D S X3 T A A

I D V T N X4 Y V V A Y Q A G D Q S Y F L R D A

P R G A E T H L F T G T T R X5 S S L P F X6 G S

Y X7 D L E R Y A G H R D Q I P L G I X8 Q L I Q

S V X9 A L R X10 P G G S T R X11 Q A R S I L I L

I Q M I S E A A R F N P I L W R X12 R Q X13 I N

S G X14 S F L P D X15 Y M L E L E T S W G Q Q S

T Q V Q H S T D G V F N N P X16 R L A I X17 X18 G

N F V T L X19 N V R X20 V I A S L A I M L F V C

G E R P S S S

Mistletoe Lectin B:

D D V T C S A S E P T V R I V G R X21 G M X22 V D

V R D D D F H D G N Q I Q L W P S K S N N D P N

Q L W T I K R D X23 T I R S N G S C L T T Y G Y

T A G V Y V M I F D C N T A V R E A T I W Q I W

X24 N G T I I N P R S N L V L A A S S G I K G T T

L T V Q T L D Y T L G Q G W L A G N D T A P R E

V T I Y G F R D L C M E S N X25 G S V W V E T C

X26 S S Q X27 N Q X28 X29 W A L Y G D G S I R P K Q N

Q D Q C L T X30 G R D S V S T V I N I V S C S X31

X32 S X33 X34 Q R W V F T N E X35 A I L N L K X36 X37

X38 X39 X40 D V A Q A N P K L R R I I I Y P A T G

K P N Q M W L P V X41

A process according to the invention for the provision of a nucleic acid molecule, which codes for the above-mentioned mistletoe lectin polypeptide in a mistletoe cell or a transgenic mistletoe plant, comprises the following steps:

a) preparing of mistletoe cell RNA or chromosomal mistletoe cell DNA and

~~b) amplifying mistletoe cell RNA or chromosomal mistletoe lectin DNA by PCR using~~ b) oligonucleotides which are derived from the mistletoe lectin polypeptide shown in Fig. 1b, ~~and~~

c) if necessary, identifying of sequences which lie 5' and 3' from the amplified nucleic acid and amplification thereof, and

d) isolating of the nucleic acid molecules amplified in step b) and/or c), and

e) if necessary, ligating of several of the nucleic acid molecules isolated in step b) and/or c), such that a nucleic acid molecule with a complete open reading frame is obtained and

f) if necessary, targeted mutation of the nucleic acid molecule obtained in order to match the nucleic acid molecule to the usual genetic code for one of the mistletoe lectin polypeptide isoforms identified in mistletoe cells and/or to optimise expression.

~~Firstly, plant RNA or DNA is isolated preferably from fresh material by various generally known processes (Quiagen experimental protocol, Nickrent D L et al., American Journal of~~

Botany, vol.81, No.9 (1994): 1149-1160, Example 1). Using the degenerate oligonucleotides BI and BII described in Example 1, which are derived from the mistletoe lectin polypeptide shown in Figure 1b, the mistletoe lectin-I gene is amplified in a PCR reaction, the conditions for which are set out in Example 2. If this amplification step does not include the complete open reading frame of ML-I, the 5' and 3' region of the amplified nucleic acids can be identified using the RACE technique with the respective oligonucleotides stated in Example 3. The nucleic acid molecules thus obtained are isolated and if necessary ligated into a vector using suitable restriction cleavage sites in such a way that this contains the complete open reading frame. A nucleic acid molecule or a fragment thereof contained in this vector, which codes for a polypeptide such as described above in a mistletoe cell or a transgenic mistletoe plant, comprises the following sequence:

1) ML-I Sequence

TACGAGAGGCTAAGACTCAGAGTTACGCATCAAACCACGGGCGAKGAATACTTCCGGTTCATCACG

CTTCTCCGAGATTATGTCTCAAGCGGAAGCTTTTCCAATGAGATACCACTCTTGCGTCAGTCTACG

ATCCCCGTCTCCGATGCGCAAAGATTTGTCTTGGTGGAGCTCACCAACCAGGGGSRRGACTCGRTY

ACGGCCGCCATCGACGTTACCAATSYKTACGTCGTGGCTTACCAAGCAGGCGACCAATCCTACTTT

TTGCGCGACGCACCACGCGGCGCGGAAACGCACCTCTTCACCGGCACCACCCGAZ1TCCTCTCTCC

CATTCAMYGGAAGCTACMCYGATCTGGAGCGATACGCCGGACATAGGGACCAGATCCCTCTCGGTA

TAGASCAACTCATTCAATCCGTCWCKGCGCTTCGTTWYCCGGGCGGCAGCACGCGTRCYCAAGCTC

GTTCGATTTTAATCCTCATTCAGATGATCTCCGAGGCCGCCAGATTCAATCCCATCTTATGGAGGK

MYCGCCAAKAYATTAACAGTGGGGMRTCATTTCTGCCAGACRTGTACATGCTGGAGCTGGAGACGA

GTTGGGGCCAACAATCCACGCAAGTCCAGCATTCAACCGATGGCGTTTTTAATAACCCAWTYCGGT

TGGCTATAYCYMCYGGTAACTTCGTGACGTTGWCYAATGTTCGCKMYGTGATCGCCAGCTTGGCGA

TCATGTTGTTTGTATGCGGAGAGCGGCCATCTTCCTCTGACGTGCGCTATTGGCCGCTGGTCATAC

GACCCGTGATAGCCGATGATGTTACCTGCAGTGCTTCGGAACCTACGGTGCGGATTGTGGGTCGAA

RTGGCATGYGCGTGGACGTCCGAGATGACGATTTCCACGATGGGAATCAGATACAGTTGTGGCCCT

CCAAGTCCAACAATGATCCGAATCAGTTGTGGACGATCAAAAGGGATRRMACCATTCGATCCAATG

GCAGCTGCTTGACCACGTATGGCTATACTGCTGGCGTCTATGTGATGATCTTCGACTGTAATACTG

CTGTGCGGGAGGCCACTATTTGGCAGATATGGGRCAATGGGACCATCATCAATCCAAGATCCAATC

TGGTTTTGGCAGCATCATCTGGAATCAAAGGCACTACGCTTACGGTGCAAACACTGGATTACACGT

TGGGACAGGGCTGGCTTGCCGGTAATGATACCGCCCCACGCGAGGTGACCATATATGGTTTCAGGG

ACCTTTGCATGGAATCAAATSRAGGGAGTGTGTGGGTGGAGACGTGCGWSAGTAGCCAAMAGAACC

AAZ2ARATGGGCTTTGTACGGGGATGGTTCTATACGCCCCAAACAAAACCAAGACCAATGCCTCAC

CKBTGGGAGAGACTCCGTTTCAACAGTAATCAATATAGTTAGCTGCAGCGSWGSWTCGKSKKSKCA

GCGATGGGTGTTTACCAATGAAKRSGCCATTTTGAATTTAAAGAVWRGSYYGRYSRTGGATGTGGC

GCAAGCAAATCCAAAGCTCCGCCGAATAATTATCTATCCTGCCACAGGAAAACCAAATCAAATGTG

GCTTCCCGTGYYMTGA

~~A nucleic acid molecule~~ according to the ~~invention or a fragment thereof, which codes for one~~ of the above-mentioned ~~MLA~~ polypeptides in a mistletoe cell or a transgenic mistletoe plant, ~~comprises the following~~ sequence.

II) MLA Sequence

TACGAGAGGCTAAGACTCAGAGTTACGCATCAAACCACGGGCGAKGAATACTTCCGGTTCATCACG

CTTCTCCGAGATTATGTCTCAAGCGGAAGCTTTTCCAATGAGATACCACTCTTGCGTCAGTCTACG

ATCCCCGTCTCCGATGCGCAAAGATTTGTCTTGGTGGAGCTCACCAACCAGGGGSRRGACTCGRTY

ACGGCCGCCATCGACGTTACCAATSYKTACGTCGTGGCTTACCAAGCAGGCGACCAATCCTACTTT

TTGCGCGACGCACCACGCGGCGCGGAAACGCACCTCTTCACCGGCACCACCCGAZ1TCCTCTCTCC

CATTCAMYGGAAGCTACMCYGATCTGGAGCGATACGCCGGACATAGGGACCAGATCCCTCTCGGTA

TAGASCAACTCATTCAATCCGTCWCKGCGCTTCGTTWYCCGGGCGGCAGCACGCGTRCYCAAGCTC

GTTCGATTTTAATCCTCATTCAGATGATCTCCGAGGCCGCCAGATTCAATCCCATCTTATGGAGGK

MYCGCCAAKAYATTAACAGTGGGGMRTCATTTCTGCCAGACRTGTACATGCTGGAGCTGGAGACGA

GTTGGGGCCAACAATCCACGCAAGTCCAGCATTCAACCGATGGCGTTTTTAATAACCCAWTYCGGT

TGGCTATAYCYMCYGGTAACTTCGTGACGTTGWCYAATGTTCGCKMYGTGATCGCCAGCTTGGCGA

~~Furthermore, a nucleic acid molecule or a fragment thereof, which codes for one of the above-~~ mentioned MLB polypeptides in a mistletoe cell or a transgenic mistletoe plant, having the ~~following sequence is made available:~~

III) MLB Sequence

GATGATGTTACCTGCAGTGCTTCGGAACCTACGGTGCGGATTGTGGGTCGAARTGGCATGYGCGTG

GACGTCCGAGATGACGATTTCCACGATGGGAATCAGATACAGTTGTGGCCCTCCAAGTCCAACAAT

GATCCGAATCAGTTGTGGACGATCAAAAGGGATRRMACCATTCGATCCAATGGCAGCTGCTTGACC

ACGTATGGCTATACTGCTGGCGTCTATGTGATGATCTTCGACTGTAATACTGCTGTGCGGGAGGCC

ACTATTTGGCAGATATGGGRCAATGGGACCATCATCAATCCAAGATCCAATCTGGTTTTGGCAGCA

TCATCTGGAATCAAAGGCACTACGCTTACGGTGCAAACACTGGATTACACGTTGGGACAGGGCTGG

CTTGCCGGTAATGATACCGCCCCACGCGAGGTGACCATATATGGTTTCAGGGACCTTTGCATGGAA

TCAAATSRAGGGAGTGTGTGGGTGGAGACGTGCGWSAGTAGCCAAMAGAACCAAZ2ARATGGGCTT

TGTACGGGGATGGTTCTATACGCCCCAAACAAAACCAAGACCAATGCCTCACCKBTGGGAGAGACT

CCGTTTCAACAGTAATCAATATAGTTAGCTGCAGCGSWGSWTCGKSKKSKCAGCGATGGGTGTTTA

CCAATGAAKRSGCCATTTTGAATTTAAAGAVWRGSYYGRYSRTGGATGTGGCGCAAGCAAATCCAA

AGCTCCGCCGAATAATTATCTATCCTGCCACAGGAAAACCAAATCAAATGTGGCTTCCCGTGYYMT

GA

The nucleotides are defined in accordance with the IUPAC-IUB code; in addition, Z_1 designates the nucleotide sequence GAT AGA or is missing, while Z_2 designates the nucleotide GGC or is missing.

~~A specific nucleic acid molecule which is to be expressed in a mistletoe cell or in a transgenic mistletoe plant and codes for ML-I, is shown in Figure 1a. Furthermore, specific nucleic acid~~

plants, which are modified in their codon usage in such a manner that as a result the expression rate is optimised.

Furthermore, the present invention makes available a process for the production of one of the above-described polypeptides, which includes the modification of sugar side-chains by enzymatic and/or chemical addition, removal and/or modification of one or several side-chains (Macindoe W M et al., Carbohydrate Research, 1995, **269** (2): 227-57; Meynial-Salles I and Combes D, J. Biotechnol., 1996, **46** (1), 1-14; Wong S Y, Current Opinion in Structural Biology, 1995, **5** (5), 599-604). In this way, the in vivo activity of individual MLA and/or MLB chains can be strengthened or weakened or in the event of any variations dependent on the expression system can be optimally matched to the natural mistletoe lectins. It is also intended that such modified mistletoe lectin can be added to a pharmaceutical composition according to the invention.

The following figures and examples illustrate the invention:

Fig.A: Representation of a mistletoe lectin-I dimer.

Fig.1: Representation of the (a) nucleic acid sequence and (b) amino acid sequence of ML-I.

Fig.2: Representation of the (a) nucleic acid sequence and (b) amino acid sequence of mistletoe lectin A1.

Fig.3: Representation of the (a) nucleic acid sequence and (b) amino acid sequence of mistletoe lectin A2.

Fig.4: Representation of the nucleic acid sequence of MLI, wherein the nucleic acid sequence is matched to the codon usage of *Brassica*.

Fig.5: Representation of the nucleic acid sequence of mistletoe lectin A1, wherein the nucleic acid sequence is matched to the codon usage of *Brassica*.

Fig.6: Representation of the nucleic acid sequence of mistletoe lectin A2, wherein the nucleic acid sequence is matched to the codon usage of *Brassica*.

Fig.7: Representation of the (a) nucleic acid sequence and (b) amino acid sequence of mistletoe lectin B.

Fig.8: Representation of the (a) nucleic acid sequence and (b) amino acid sequence of mistletoe lectin B1.

Fig.9: Representation of the (a) nucleic acid sequence and (b) amino acid sequence of

mistletoe lectin B2.

Fig.10: Representation of the (a) nucleic acid sequence and (b) amino acid sequence of mistletoe lectin B3.

Fig.11: Representation of the (a) nucleic acid sequence and (b) amino acid sequence of mistletoe lectin B4.

Fig.12: Representation of the (a) nucleic acid sequence and (b) amino acid sequence of mistletoe lectin B5.

Fig.13: Representation of the nucleic acid sequence of mistletoe lectin B, wherein the nucleic acid sequence is matched to the codon usage of *Brassica*.

Fig.14: Representation of the nucleic acid sequence of mistletoe lectin B1, wherein the nucleic acid sequence is matched to the codon usage of *Brassica*.

Fig.15: Representation of the nucleic acid sequence of mistletoe lectin B2, wherein the nucleic acid sequence is matched to the codon usage of *Brassica*.

Fig.16: Representation of the nucleic acid sequence of mistletoe lectin B3, wherein the nucleic acid sequence is matched to the codon usage of *Brassica*.

Fig.17: Representation of the nucleic acid sequence of mistletoe lectin B4, wherein the nucleic acid sequence is matched to the codon usage of *Brassica*.

Fig.18: Representation of the nucleic acid sequence of mistletoe lectin B5, wherein the nucleic acid sequence is matched to the codon usage of *Brassica*.

Example 1

Mistletoe plants of the species *Viscum album L. spp. platyspermum* Kell were harvested from poplars growing in Alsace and frozen directly after harvesting. The plant material was crushed in liquid nitrogen in the laboratory and then the DNA from 100 mg of plant material was isolated by the process described in the Qiagen DNeasy Plant Mini-Handbook 09/96.

Example 2

PCR Conditions for the Amplification of Mistletoe Lectin-I DNA

For the amplification of genomic mistletoe lectin-I DNA, 100 ng of template DNA, prepared as stated in Example 1, were used in a PCR process with 30 cycles using Taq polymerase (Boehringer Mannheim). 1 µg of primer, $MgCl_2$ (end concentration 2 mM), nucleotide mixture A, T, C, G (end concentration 0.2 mM) and 2.5 units of Taq polymerase were added

to the template DNA. The reaction was started as hot-start PCR by a denaturation step of the DNA for 5 minutes at 94°C. In this, the enzyme and the remaining reagents only mixed after a wax barrier between the components had melted. The 30 subsequent cycles are performed under the following conditions:

Denaturation:	94°C	30 seconds
Annealing:	55°C	30 seconds
Amplification:	72°C	1 minute.

Following the 30 cycles, a 7-minute elongation reaction at 72°C was also performed, before the reaction mixture was cooled down to 4°C.

The primers used in the PCR process hybridised with fragments of the genomic DNA coding for MLB chain DNA and had the following sequences:

B1 GTN MGN GAY GAY GAY TTY CA

B2 AT YTG RTT NGG YTT NCC NGT

The nucleotides are defined in accordance with the IUPAC-IUB code.

The oligonucleotide B1 hybridised to the nucleic acid region that corresponds to amino acids 24 to 30 of the MLB sequence, while the oligonucleotide B2 hybridised to the complementary DNA sequence coding for amino acids 253-258 of MLB.

Example 3

In order to determine the flanking 3' and 5' sequences of the DNA amplified in Example 2, the RACE technique was used. 2 μg of RNA template in cDNA synthesis buffer (end concentration: 20 mM Tris-HCl, 8 mM $MgCl_2$, 30 mM KCl, 1 mM dithiothreitol; pH 8.5 (20°C)) were treated with AMV reverse transcriptase, the deoxynucleotides and the specific primer (see below) and incubated for 60 mins at 65°C. Next, the sample was incubated for 10 mins at 65°C. After the purification of the first cDNA strand, the "tailing" reaction was carried out with 2/5 of the synthesised cDNA with terminal transferase. After the tailing reaction, a PCR was performed with the oligo-dT anchor primer and the specific primer (see above for incubation conditions, except for the annealing temperature, which was lowered to

50°C). For the determination of the 5' regions of the nucleic acid molecules amplified in Example 2, the oligonucleotide having the following sequence was used:

CAC AGC AGT ATT ACA GTC GAA.

B25

A DNA sequence complementary to this oligonucleotide codes for the amino acid sequence 79-85 of the MLB polypeptide. In order to determine the 3' regions of the amplified nucleic acid molecules, the oligonucleotide having the following sequence was used in a similar experiment:

GTC TAT GTG ATG ATC TTC GAC TGT.

B24

This nucleic acid sequence codes for the amino acid region 74-81 of the MLB polypeptide. For the 3' RACE reaction, the same incubation conditions as for the 5' RACE were used, except for the "tailing" reaction, which is not necessary here because of the polyA tail of the mRNA. In both processes, the oligo-dT anchor primer of the Boehringer Mannheim kit was used.

Example 4

Pharmaceutical Composition with Cytotoxic Action:

Mistletoe, tobacco and rape cells are transfected with RNA vectors which code for MLA1 and MLA2, the respective cells are harvested after a few days, and the MLA1 and MLA2 proteins purified by affinity chromatography. As gel material, divinylsulphone (DVS)-activated lactose-coupled Sepharose 4B (Pharmacia) is used. By treatment with 0.2 M HCl, the material is activated, i.e. the Sepharose structure is partially hydrolysed and sugar-binding sites to which the lectins can bind are freed. 100 ml of gel material are washed with 0.2 M HCl in a Buchner funnel and suspended in 200 ml of 0.2 M HCl. The hydrolysis of the gel material is effected by 3.5-hour incubation of the suspension at 50°C in the water-bath. The suspension is washed free of acid with water and then with peptide eluent (0.05 K_2HPO_4x $3H_2O$, 0.15 M NaCl, pH 7.0). Then the suspension is degassed, the peptide eluent removed by suction, and the viscous liquid gel material filled into an empty column XK50/30 (3 x 50 cm, Pharmacia) and packed with peptide eluent pH 7.0 at a flow rate of 2.5 ml/min initially and then 5 ml/min. The column is equilibrated with the same eluent at a flow rate of 1 ml/min. The cell extract obtained from the transfected mistletoe cells is centrifuged and the supernatant loaded onto the column. The separation is performed at a flow rate of 1 ml/min with peptide

eluent pH 7.0. The lectins are eluted from the column material with a buffer of 0.2 M lactose in peptide eluent pH 7.0 at a flow rate of 2 ml/min. The elution of the lectin from the column is measured by determination of the absorption at 206 nm. The lectin-containing fractions are collected, frozen and lyophilised. If desired, a further purification step on an HPLC column can be performed. Suitable for this is a Vydac C4 300 A column, which is run at a flow rate of 300 µl/min and a gradient of 20% to 100% B in 60 minutes, where eluent A is 0.17% TFA in water and eluent B is 0.15% TFA in 80% CH_3CN in water. The elution of the mistletoe lectins is detected at a wavelength of 214 nm.

The purified MLA-1 and MLA-2 polypeptides are coupled to a suitable cell recognition molecule. If the cell recognition molecule is a mono- or polyclonal antibody, this can for example be bound to the cytotoxic MLA1 or MLA2 using glutaraldehyde or be directly expressed as chimaeric fusion protein (antibody-MLA) in the appropriate expression system.

Example 5

Pharmaceutical Composition:

Mistletoe cells are transfected with RNA vectors which code for the mistletoe lectins MLA1 and MLA2 and mistletoe lectins MLB to MLB6. After a few days, the mistletoe lectin monomers or dimers are extracted from the mistletoe cells and purified by processes such as are described in Example 4. The monomers thus obtained can be fused in vitro to heterologous and homologous dimers. In this way, a large number of different combinations of the individual MLA and MLB polypeptides are formed. The heterogeneous mixture of ML-1 dimers and monomers thus produced is lyophilised and used for formulation with a suitable vehicle.